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# Small tropical forest trees have a greater capacity to adjust carbon metabolism to long-term drought than large canopy trees

Running Head: Small tree carbon metabolism drought responses

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## Abstract

The response of small understory trees to long-term drought is vital in determining the future composition, carbon stocks and dynamics of tropical forests. Long-term drought is, however, also likely to expose understory trees to increased light availability driven by drought-induced mortality. Relatively little is known about the potential for understory trees to adjust their physiology to both decreasing water and increasing light availability. We analysed data on maximum photosynthetic capacity ( $J_{\max}$ ,  $V_{\max}$ ), leaf respiration ( $R_{\text{leaf}}$ ), leaf mass per area (LMA), leaf thickness and leaf nitrogen and phosphorus concentrations from 66 small trees across 12 common genera at the world's longest running tropical rainfall exclusion experiment and compared responses to those from 61 surviving canopy trees. Small trees increased  $J_{\max}$ ,  $V_{\max}$ ,  $R_{\text{leaf}}$  and LMA (71%, 29%, 32%, 15% respectively) in response to the drought treatment, but leaf thickness and leaf nutrient concentrations did not change. Small trees were significantly more responsive than large canopy trees to the drought treatment, suggesting greater phenotypic plasticity and resilience to prolonged drought, although differences among taxa were observed. Our results highlight that small tropical trees have greater capacity to respond to ecosystem level changes and have the potential to regenerate resilient forests following future droughts.

**Key words:** drought, leaf respiration, light, ontogeny, photosynthesis, through-fall exclusion experiment, tropical forest, understory.

## Introduction

Climate change can simultaneously affect multiple environmental variables across ecosystems globally (IPCC, 2019). However, little is known about how trees respond to multiple environmental shifts, especially in tropical forests (Niinemets, 2010; Bonal *et al.*, 2016). Both episodic and sustained droughts have been shown to cause mortality of large canopy trees in Amazonia (Nepstad *et al.*, 2007; Phillips *et al.*, 2010; Bennett *et al.*, 2015; Meir *et al.*, 2015; Rowland *et al.*, 2015a), resulting in canopy openings and changes in the understory environment (Chazdon & Fetcher, 1984; Brown, 2009; Pfeifer *et al.*, 2016; Rowland *et al.* under review). Small understory trees must therefore respond to concurrent reductions in soil moisture availability and increased light availability to survive, compete and grow under drought conditions. The ability of these small trees to adjust their physiology in response to multiple environmental shifts could be critical for predicting the future of tropical forests.

If canopy trees are susceptible to mortality during drought under future climates, it becomes important to understand the response of understory trees to drought conditions within higher light environments, and concomitant changes in air temperature and VPD. However, most of our current knowledge of tropical tree drought responses is focused on either large canopy trees or seedlings (e.g. Poorter & Hayashida-Oliver, 2000; Nepstad *et al.*, 2007; Schuldt *et al.*, 2011; O'Brien *et al.*, 2014; Rowland *et al.*, 2015b; Feldpausch *et al.*, 2016; O'Brien *et al.*, 2017). Some studies have investigated the impacts of short-term drought events on tropical understory trees (e.g. Phillips *et al.*, 2010; Newbery *et al.*, 2011), but, to our knowledge, no studies to date have investigated the effects of prolonged drought in tropical forests on understory trees also exposed to elevated light conditions. This represents a gap in our knowledge as understory trees tend to have very different resource requirements to adult trees and seedlings (Sterck *et al.*, 2014). If we are to fully predict the fate of

tropical forests and improve climate predictions from the latest generation of demography-based vegetation models, it is critical to understand the physiological responses of understory trees in drought-affected forests (Moorcroft *et al.*, 2001; Smith *et al.*, 2008; Fisher *et al.*, 2018; Moore *et al.*, 2018).

Differences in physiology, microenvironments and resource requirements will likely lead to different drought responses in small understory trees compared with large canopy trees (Kitajima & Poorter, 2008). Small trees tend to be particularly sensitive to reductions in soil moisture availability (Ruger *et al.*, 2012; Kitajima *et al.*, 2013; Quevedo-Rojas *et al.*, 2018), because of smaller total carbon reserves (Hartmann *et al.*, 2018) and shallower rooting depths (Stahl *et al.*, 2013; Brum *et al.*, 2018). However, vulnerability to drought may actually be lower in small trees, as carbon reserves per unit biomass may be greater (Hartmann *et al.*, 2018). An ability to maintain a positive balance between carbon assimilation and use will ultimately be critical for these small trees to survive and avoid carbon starvation under drought conditions (O'Brien *et al.*, 2014; McDowell *et al.*, 2018). The adjustment of functional traits related to carbon metabolism, including maximum photosynthetic capacity ( $J_{\max}$  and  $V_{\max}$ ) and leaf respiration ( $R_{\text{leaf}}$ ), is important for buffering long-term reductions in soil moisture availability. Under drought stress, some trees have been shown to reduce photosynthetic capacity because of drought-induced impairment or for nutrient re-allocation for stress repair (Damour *et al.*, 2008; Damour *et al.*, 2009), whilst others have been shown to maintain photosynthetic capacity to optimise carbon assimilation during wetter periods (Rowland *et al.*, 2015b). In response to this,  $R_{\text{leaf}}$  can increase under drought conditions to support stress-related repair or support osmoregulation (Rowland *et al.*, 2015b), however perhaps more typically  $R_{\text{leaf}}$  has been found to decrease during drought stress in response to reduced photosynthesis (Atkin & Macherel, 2009; Ayub *et al.*, 2011). The extent of plasticity in these carbon metabolic traits could determine the likelihood of small trees

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surviving long periods of reduced soil moisture availability. However, these traits are not simply controlled by drought stress, but are also highly sensitive to light availability (Poorter *et al.*, 2009; Atkin *et al.*, 2015; Hasper *et al.*, 2017). Consequently, the light environment of a tree should also be considered when understanding responses to drought.

Tropical forests display strong vertical gradients in light availability with small understory trees generally adapted to shade conditions relying on diffuse light and unpredictable, fleeting sunflecks for the majority of their carbon assimilation (Chazdon & Pearcy, 1991; Leakey *et al.*, 2003). Under normal conditions, tropical trees are able to acclimate their leaf physiology to the steep gradient in irradiance experienced through the vertical profile of the canopy, with photosynthetic capacity and LMA increasing with light availability (Meir *et al.*, 2002; Poorter *et al.*, 2009; Cavaleri *et al.*, 2010; Domingues *et al.*, 2010). Trees modify their leaf physiology to optimise the balance between carbon gain and carbon and water loss, in order to maximise growth, reproduction and competitiveness. Both large and small understory trees in tropical forests have been shown to be highly responsive and plastic to changes in their light environment (Kitajima *et al.*, 2013; Quevedo-Rojas *et al.*, 2018), suggesting light is likely to be the most limiting factor for photosynthesis in intact tropical forests. However, if another factor, such as water availability, also becomes limiting, these trees may no longer be able to acclimate to high light levels, potentially leading to negative impacts of increasing light on growth and survival. Increases in light availability under drought conditions may result in excessive photon flux density, elevated leaf temperatures and elevated VPD, inducing photoinhibition (Kamaluddin & Grace, 1992; Mulkey & Pearcy, 1992; Krause *et al.*, 1995), prolonged stomatal closure (Reynolds-Henne *et al.*, 2010) and xylem embolism. To avoid these negative consequences of concurrent high light and drought stress, small understory trees may need to modify their physiology in different ways compared to canopy trees.

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Insights from the same eastern Amazon throughfall exclusion experiment used in this study showed the responses of large trees to drought were indeed influenced by the light environment (Rowland et al. under review). Large trees that had also experienced increased canopy exposure following mortality events, experienced reductions in photosynthetic capacity under drought conditions, whilst those that did not maintained photosynthetic capacity (Rowland et al. under review). However, large trees will have experienced relatively minor shifts in their light environment when compared to small understory trees. These larger trees may also be more exposed to hydraulic stress from the drought, which may limit their ability to respond positively to elevated light (Bittencourt *et al.*, 2020), although they may already be acclimated to higher VPD conditions at the top of the canopy so may not experience hydraulic stress from sudden shifts in VPD that will occur in the understory. Studying how small understory trees adjust their leaf physiology to concurrent shifts in water and light availability and how these responses differ from large canopy trees may also give new insights into the potential for forest regeneration following drought events.

Here, we use data from a 15-year tropical forest drought experiment located in eastern Amazonia, to test how long-term drought affects carbon metabolism and leaf morphology in small understory trees. By comparing data on maximum photosynthetic capacity ( $J_{\max}$  and  $V_{\max}$ ), leaf dark respiration ( $R_{\text{leaf}}$ ), leaf mass per area and leaf thickness from 66 small understory trees (1-10 cm DBH) against those from 61 surviving canopy trees across 12 genera between a throughfall exclusion (TFE) experiment and neighbouring control plot, we test the following hypotheses:

- 1) Small understory trees respond to canopy openings following long-term drought stress by increasing photosynthetic capacity ( $J_{\max}$  and  $V_{\max}$ ) in response to elevated light availability.



- 2) Small understory trees increase leaf dark respiration and LMA in response to long-term drought stress.
- 3) Leaf physiological traits ( $J_{\max}$ ,  $V_{\max}$ ,  $R_{\text{leaf}}$  and LMA) are more responsive to reduced soil moisture availability and canopy openings following prolonged soil moisture deficit in small understory trees than large trees.

## Methods

### *Study site*

This study was carried out at the world's longest running TFE experiment in Caxiuanã National Forest Reserve, Para, Brazil (1°43'S, 51°27'W). The experiment is located in seasonally dry *terra firme* forest with an annual precipitation of 2000-2500 mm. Here, a pronounced dry season occurs between June and November, where average precipitation drops to <100 mm per month. The experiment consists of two plots: the TFE plot (1 ha) where 50% of incoming canopy throughfall has been excluded using clear plastic panels at 1-2 m height since 2002, and a neighbouring control plot (1 ha) located <50 m from the TFE. The two plots were both trenched around the perimeter to a depth of 1-2 m to minimise horizontal throughflow. Both plots have been continuously maintained and monitored since 2001. For experimental details, see (Fisher *et al.*, 2007; da Costa *et al.*, 2010; Rowland *et al.*, 2015b; Meir *et al.*, 2018). Reduced soil moisture availability on the TFE plot compared with the control plot has been shown previously with lower mean pre-dawn leaf water potentials on the TFE plot (SI Fig. 1) (Bittencourt *et al.*, 2020).

### *Sampling*

From August to September 2017, we sampled 66 small trees (1-10 cm diameter at breast height; 1.3m DBH; 2.7-23.0 m height) across the two plots: 30 from the TFE and 36 from the control. We selected individuals from 12 of the most common genera within the two plots (*Duguetia*, *Eschweilera*, *Inga*, *Iryanthera*, *Licania*, *Manilkara*, *Minquartia*, *Ocotea*, *Protium*, *Tetragastris*, *Swartzia*, *Vouacapoa*) in accordance with a corresponding study on large tree carbon metabolism and storage (SI Table 1a; Rowland *et al.* under review). All species represent canopy or emergent trees when mature. In order to minimise edge effects within the plots, we sampled trees located within one quarter of each 1 ha

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plot (i.e. 0.25 ha), with all trees located at least 20 m from the plot perimeter. For each genus, individual tree selection was designed to cover a range of sizes from 1 to 10 cm DBH. Two people independently assessed the relative canopy position of each tree and assigned it as either shaded or in a canopy gap according to whether the leaves experienced vertical shading or not. All canopy position assessments were recorded at approximately the same time each day, and the presence of leaves directly above the target tree were used to minimise bias from different sun positions at the time of measurement.

We selected an additional 61 large trees (>20 cm DBH: range 20.2-67.9 cm) from a parallel study across eight corresponding genera (*Eschweilera*, *Inga*, *Licania*, *Manilkara*, *Minquartia*, *Protium*, *Swartzia*, *Tetragastris*) to compare responses of large trees against a subset of 48 small trees for the same eight genera (SI Table 1b). For more details about large tree sampling, see Rowland et al. (under review).

#### *Gas exchange measurements*

During the peak dry season (August-September 2017), we collected a branch of approximately 1 m length from the top of the crown of each tree, using pole pruners. For the majority of the trees (> 95%), the branches were cut between 09:00 and 10:00 hrs, but on some occasions branches were cut between 10:00 and 13:00. Once harvested, branches were immediately placed in water and were cut twice underwater to restore water supply to the leaves (Domingues *et al.*, 2010). The branches were subsequently left to stabilise in full sunlight for a minimum of 30 minutes. Following stabilisation, we selected non-senescing, fully formed leaves to be measured using two cross-calibrated portable photosynthesis systems (LI-6400XT and LI-6800, LI-COR, Nebraska, USA). For each tree, we measured one leaf for estimates of photosynthetic capacity and one neighbouring leaf for dark-adapted leaf respiration ( $R_{leaf}$ ).

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We performed photosynthetic CO<sub>2</sub> response curves (A-C<sub>i</sub>) to estimate maximum photosynthetic capacity. We placed a leaf within the leaf chamber of a portable photosynthesis system and measured net photosynthetic assimilation (A) and leaf internal carbon dioxide (CO<sub>2</sub>) concentration (C<sub>i</sub>). We generated A-C<sub>i</sub> curves by manipulating CO<sub>2</sub> concentrations within the leaf chamber (400, 200, 75, 400, 800, 1200, 2000 ppm), whilst providing a photosynthetic active radiation (PAR) of 1500 μmol m<sup>-2</sup> s<sup>-1</sup>, a temperature of 28 °C and a relative humidity of 60-70%. Light response curves carried out on a subset of the samples (n = 8) ensured 1500 μmol m<sup>-2</sup> s<sup>-1</sup> PAR represented saturating light conditions for the leaves (data not shown). In order to maintain data quality, A-C<sub>i</sub> curves were aborted if stomatal conductance (g<sub>s</sub>) dropped below 0.03 mol m<sup>-2</sup> s<sup>-1</sup> (following Rowland *et al.*, 2015b). No difference between measurements of photosynthetic capacity or R<sub>leaf</sub> on cut versus uncut branches have been found in previous studies at this site (Rowland *et al.*, 2015b), suggesting our measurements reflect true values of *in situ* leaves and differences between the two plot treatments. We waited for steady-state conditions within the leaf chamber to be reached before any gas exchange measurements were recorded. Using these data, we estimated the maximum rate of electron transport (J<sub>max</sub>) and the maximum rate of carboxylation (V<sub>cmax</sub>) standardised to 25 °C following the equations from the C<sub>3</sub> photosynthesis model of Farquhar *et al.* (1980) as in Sharkey *et al.* (2007) using the *optim* function from the *stats* package in R statistical software (R Core R Core Team, 2013). Ten trees were removed from our sample where A-C<sub>i</sub> curves could not be fitted.

Dark-adapted leaf respiration (R<sub>leaf</sub>) was measured on a leaf adjacent to that used to measure maximum photosynthetic capacity. Leaves were wrapped in aluminium foil for a minimum of 30 minutes before the portable photosynthesis system was used to measure R<sub>leaf</sub>. We maintained stable leaf chamber conditions of 400 ppm CO<sub>2</sub> concentration, 0 μmol m<sup>-2</sup> s<sup>-1</sup> PAR and 28 °C for all respiration measurements. Following stabilisation within the leaf chamber, three measurements of respiration

were recorded at five second intervals, then standardised to 25 °C using a Q10 value of 2.2 following Rowland *et al.* (2015b) and a mean value was calculated.

#### *Leaf morphological traits*

After completing leaf gas exchange measurements, we removed each of the leaves used to measure  $A-C_i$  curves and  $R_{\text{leaf}}$  from the branch and placed them in a sealed airtight plastic zip-lock bag. Moist cotton wool was placed in the bag to maintain high levels of humidity and prevent any water loss from the leaf. We scanned leaves using a flatbed scanner (CanoScan LiDE 120, Canon Inc., Tokyo, Japan) and calculated the area of each leaf using ImageJ software (Schneider *et al.*, 2012). Leaves were then placed in an oven to dry for 24 hours at 70 °C, to constant mass. We measured dry leaf mass using a precision balance and calculated leaf mass per area (LMA,  $\text{g m}^{-2}$ ) by dividing dry leaf mass by leaf area. We used LMA from the leaves used to measure  $A-C_i$  curves and  $R_{\text{leaf}}$  to estimate maximum photosynthetic capacity and  $R_{\text{leaf}}$ , respectively, on a mass basis. We measured leaf thickness at three different points on fully hydrated leaves using digital callipers avoiding any major veins and calculated the mean. We averaged the LMA and thickness for the  $A-C_i$  and  $R_{\text{leaf}}$  leaves to generate an estimate for the overall branch.

#### *Leaf nutrient analyses*

We collected an additional sample, depending on leaf size, of 3-20 leaves adjacent to the leaves used for the gas exchange measurements to quantify leaf nitrogen and phosphorus concentrations ( $[N]_{\text{leaf}}$ ,  $[P]_{\text{leaf}}$ ). Following collection, leaves were dried in an oven for 24 hours at 70 °C to constant mass before being combined with the leaves used for gas exchange measurements. Major veins were then removed and the remaining foliar matter was ground to a fine powder using a ball mill. Nitrogen concentrations were measured using the semi-micro Kjeldahl method (Malavolta, 1997), whilst

phosphorus concentrations were measured using a Femto 600+ Spectrophotometer using the ammonium metavanadate method (Malavolta, 1997). All analyses were tested against laboratory standards. We divided the gas exchange parameters on a mass basis by leaf nutrient concentrations to estimate leaf photosynthetic and respiratory nutrient use efficiency.

#### *Data analysis*

##### *Small tree physiological responses*

We used linear mixed effects models, using the package *lme4* (Bates *et al.*, 2014), to test for treatment (TFE vs control) and taxonomic effects on leaf gas exchange, morphological, nutrient content and nutrient-use efficiency traits in small trees ( $n = 66$ ). Taxonomy was included as a random intercept effect in our models, whilst the TFE treatment was incorporated as a fixed effect. We tested for a genus and a species nested within genus taxonomic effect by comparing the full linear mixed effects model to a generalised least squares model, following Zuur *et al.* (2009) using the *nlme* package (Pinheiro *et al.*, 2012). When genus was not significant, linear models were used to test the significance of the fixed effects. For each trait response variable, the treatment effect was tested by comparing the Akaike Information Criterion corrected for sample size (AICc) between models using restricted maximum likelihood in the package *MuMIn* (Bartón, 2018).

Since traits may be coordinated, we additionally used standardised major axis regression (SMA) to test the effect of the TFE trait trade-offs. We tested for differences in the slopes and intercepts of the bivariate trait-trait relationships between small trees on the different treatments using a Wald test in the package *smatr* (Warton *et al.*, 2012). We investigated relationships between the gas exchange measurements and leaf morphological variables and the relationship between  $J_{\max}$  and  $V_{\max}$ .

### *Small and large tree comparisons*

We tested for differences in individual tree-level responses to the TFE treatment for large canopy (n = 61) and small understory trees (n = 48), for the same set of species and genera in which data were available (SI Table 1b). We used linear mixed effect models to test the effect of the TFE treatment, tree size (large vs small), canopy shading (canopy gap vs fully shaded) and the interactions between treatment and tree size, and between treatment and canopy shading on leaf gas exchange and morphological traits. We also tested for taxonomic effects by including genus and species nested within genus as random effect variables and comparing to a generalised least squares model, following the same protocol used for small tree analyses (Bartón, 2018). Within this paper, all data represent the mean and associated errors denote standard errors of the mean. All data analyses were undertaken on individual tree-level data in R (R 3.5.1, R Core Team 2018).

## Results

### *Physiological responses to long-term drought in small understory trees*

We found a significant positive effect of the TFE, relative to the control plot, on both mean  $J_{\max}$  (71.1%;  $\Delta 14.18 \pm 2.65 \mu \text{mol m}^{-2} \text{s}^{-1}$ ,  $P < 0.001$ ; Fig. 1a) and  $V_{\text{cmax}}$  (29.2%;  $\Delta 3.99 \pm 1.40 \mu \text{mol m}^{-2} \text{s}^{-1}$ ,  $P < 0.01$ ; Fig. 1b) standardised to 25°C.  $J_{\max}$  ranged from 7.11 to 41.00  $\mu \text{mol m}^{-2} \text{s}^{-1}$  in the control trees compared with 15.94 to 68.93  $\mu \text{mol m}^{-2} \text{s}^{-1}$  in the TFE trees, whilst  $V_{\text{cmax}}$  ranged from 7.84 to 26.12  $\mu \text{mol m}^{-2} \text{s}^{-1}$  in the control and 11.52 to 39.19  $\mu \text{mol m}^{-2} \text{s}^{-1}$  in the TFE. We found a 32.2% increase in 25 °C standardised leaf dark respiration ( $R_{\text{leaf}}$ ) on the TFE plot compared to the control plot ( $\Delta 0.12 \pm 0.06 \mu \text{mol m}^{-2} \text{s}^{-1}$ ,  $P = 0.045$ ; Fig. 1c), with values ranging from 0.11-0.95  $\mu \text{mol m}^{-2} \text{s}^{-1}$  on the control and 0.06-1.49  $\mu \text{mol m}^{-2} \text{s}^{-1}$  on the TFE. Leaf mass per area (LMA) was  $10.68 \pm 4.04 \text{ g m}^{-2}$  (15.1%) higher for small trees found on the TFE plot ( $P < 0.01$ ; Fig. 1d), but mean leaf thickness did not significantly differ between the two treatments (Table 1). We found no significant differences in mean leaf nitrogen and phosphorus concentrations ( $[N]_{\text{leaf}}$  and  $[P]_{\text{leaf}}$ ) on a mass-basis between the control and TFE treatments (Fig. 1 e-f, Table 1). We found a significant effect of genus on the intercept for all of the traits except  $J_{\max}$  and  $R_{\text{leaf}}$ , but no significant species-nested-within-genus effect for any traits (SI Table 2).

Using standardised major axis (SMA) regression to compare differences in the relationships between  $J_{\max}$ ,  $V_{\text{cmax}}$ ,  $R_{\text{leaf}}$ , and LMA across the two experimental plots, we found no significant trait-trait relationships, except for  $J_{\max}$ - $V_{\text{cmax}}$  (SI Table 3). An increase in the  $\log_{10}$ -transformed electron transport rates relative to carboxylation on the TFE treatment compared with the control was observed (TFE =  $1.72 \pm 0.07$ , Control =  $1.36 \pm 0.08$ , Wald = 8.503, df = 1,  $P = 0.004$ ; Fig. 2), but no difference in the slope of the relationship ( $P = 0.757$ ).



We found no relationship between  $J_{\max}$ ,  $V_{\max}$  or  $R_{\text{leaf}}$  with  $[N]_{\text{leaf}}$  or  $[P]_{\text{leaf}}$  on either plot (SI Table 3). However, the TFE trees significantly increased  $J_{\max}$  on a mass basis per unit  $N_{\text{leaf}}$  and unit  $P_{\text{leaf}}$  relative to the control (Fig. 3).  $J_{\max}$  increased per unit nitrogen and unit phosphorus in the TFE trees by  $0.006 \pm 0.002 \mu\text{mol g}^{-1} \text{s}^{-1}$  and  $0.227 \pm 0.068 \mu\text{mol g}^{-1} \text{s}^{-1}$ , respectively ( $J_{\max}/N_{\text{leaf}}$ :  $P = 0.018$ ;  $J_{\max}/P_{\text{leaf}}$ :  $P < 0.01$ ; Fig. 3). Overall, neither  $V_{\max}$  nor  $R_{\text{leaf}}$  increased per unit  $N_{\text{leaf}}$  or  $P_{\text{leaf}}$  in the TFE trees relative to the control (Fig. 3).

#### ***Comparison of responses to long-term drought between large canopy and small understory trees***

We compared the responses of leaf traits in large (>20 cm DBH) and small (1-10 cm DBH) trees to the TFE treatment and to canopy shading using linear mixed models. We found a significant effect of tree size and the TFE treatment on  $J_{\max}$ ,  $V_{\max}$ ,  $R_{\text{leaf}}$ , and LMA, except for a non-significant effect of the TFE treatment on  $V_{\max}$  and  $R_{\text{leaf}}$  (Fig. 4; Table 1).  $J_{\max}$ ,  $V_{\max}$ , and  $R_{\text{leaf}}$  were, respectively, 33.8% ( $\Delta 18.82 \pm 3.01 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), 25.3% ( $\Delta 7.59 \pm 2.18 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and 36.5% ( $\Delta 0.23 \pm 0.05 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) lower in small trees after accounting for canopy shading in the models. Canopy shading had a significantly negative effect on  $J_{\max}$  and  $V_{\max}$ , but not  $R_{\text{leaf}}$  or LMA, with maximum photosynthetic capacity higher for trees positioned in the canopy or a canopy gap compared to trees shaded by the canopy (Table 1). In addition, a significant interaction between the TFE treatment and tree size for  $J_{\max}$  and  $V_{\max}$  was found, showing the response of maximum photosynthetic capacity to the TFE treatment to be dependent on tree size after accounting for canopy shading. The TFE effect on small tree  $J_{\max}$  and  $V_{\max}$  was reduced compared to large trees by 100.3% ( $\Delta 18.88 \pm 4.56 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) and 88.1% ( $\Delta 6.69 \pm 2.58 \mu\text{mol m}^{-2} \text{s}^{-1}$ ), respectively. We found no significant effect of canopy shading, TFE treatment or tree size on leaf thickness. A significant genus effect was present for all leaf traits except  $R_{\text{leaf}}$ , showing these leaf traits are not solely determined by the environment, but also vary with taxonomy. For

example, *Inga* and *Swartzia* had the highest  $J_{\max}$  and  $V_{\max}$ , respectively, whilst *Protium* had the lowest  $J_{\max}$  and  $V_{\max}$  of the genera studied, according to the intercepts in our mixed effect models. See Table 1 and SI Table 4 for effect sizes, standard errors, significance and  $R^2$  values.

When the above results were repeated separately for the five most common genera in our dataset, we discovered the effect of tree size and the TFE treatment varied for different genera (Fig. 5). We show *Eschweilera* and *Swartzia* to have a positive response to the TFE treatment in small trees for  $J_{\max}$ , with *Swartzia* having the largest (positive) response for  $V_{\max}$  (Fig. 5). In contrast our data show *Inga* and *Protium* to exhibit little response to the TFE treatment in small trees for all traits. Different genera also exhibit different degrees of variation in their leaf morphology, showing that the responses to the TFE treatment vary by taxa (Fig. 5). Despite relatively small sample sizes, our data indicate similar or greater intraspecific than interspecific variation in leaf traits of small trees, especially photosynthetic capacity on the TFE, with the standard deviation in trait values within species exceeding the standard deviation in species-level means on the TFE in 8/9, 6/9, 4/10, and 2/10 species for  $J_{\max}$ ,  $V_{\max}$ ,  $R_{\text{leaf}}$  and LMA respectively (SI Table 5).

## Discussion

We demonstrate that small understory trees (1-10 cm DBH) are more responsive than large canopy trees (>20cm DBH) to prolonged drought conditions. Canopy openings driven by drought-induced mortality of large trees (Rowland *et al.*, 2015a) have allowed small understory trees to increase photosynthetic capacity ( $J_{\max}$  &  $V_{\max}$ ), leaf respiration ( $R_{\text{leaf}}$ ) and leaf mass per area (LMA) in spite of reduced soil moisture availability and no changes in leaf nutrient concentrations (Fig. 1 & SI Fig. 1 & 2). These small understory trees show greater capacity to adjust their leaf physiology than large canopy trees following 15 years of through-fall exclusion (TFE; Fig. 4), suggesting responses to drought are dependent on tree size. Measured values of  $J_{\max}$ ,  $V_{\max}$ ,  $R_{\text{leaf}}$  and LMA in this study remained lower in small trees compared to large trees for both treatments, but values for small understory trees under the TFE treatment approach those of large trees for  $J_{\max}$  and  $V_{\max}$  after accounting for canopy position (canopy gap vs shaded understory) in our statistical models (Table 1). This indicates small trees are sufficiently plastic to increase photosynthetic capacity with higher light availability, even under drought conditions.

Maximum photosynthetic capacity was highly responsive to the TFE treatment in small understory trees with a 71% increase in the maximum rate of electron transfer ( $J_{\max}$ ) and a 29% increase in the maximum rate of carboxylation ( $V_{\max}$ ; Fig. 1). The increase in light availability in the droughted forest and the release from extreme light limitation is likely to be driving these physiological adjustments as light represents an important control on both traits (Hasper *et al.*, 2017). Typically, very little light penetrates the canopy to the understory in tropical forests because of the high leaf area index characteristic of tropical rainforests (Asner *et al.*, 2003). With the opening of the canopy, increased light availability may mean understory trees are no longer dependent on sunflecks for the majority of

their photosynthesis (Chazdon & Pearcy, 1991; Leakey *et al.*, 2003). As a consequence, the magnitude of the change in the light environment may be sufficiently great that light acts as a strong selection pressure on photosynthetic capacity in these small trees, even when exposed to long-term drought conditions.

The greater plasticity of  $J_{\max}$  compared to  $V_{\max}$  and resultant increase in the  $J_{\max} / V_{\max}$  ratio on the TFE plot (Fig. 2) is highly indicative of a response to light, as the electron transfer reactions are directly involved in capturing light energy (Farquhar *et al.*, 1980; Sharkey *et al.*, 2007). A similar shift in the ratio in tropical montane cloud forests compared to lowland tropical forests has been attributed to lower total daily light availability caused by cloud cover, but with periods of intermittent intense light (van de Weg *et al.*, 2012). Following the mortality of canopy trees, the understory may experience similar sporadic periods of intense light, as a consequence of increased sunfleck occurrence and duration. A larger upregulation of  $J_{\max}$  compared to  $V_{\max}$  may allow these trees to maximise light capture during these prolonged sunflecks, with carboxylation reactions occurring subsequently (Pearcy, 1990). The division of the light dependent and light independent reactions may allow small understory trees to increase overall carbon assimilation whilst avoiding the additional maintenance costs of elevated  $V_{\max}$ .

We show small trees can increase  $J_{\max}$ , but not  $V_{\max}$ , without additional leaf nitrogen or phosphorus (Fig. 1), most likely by increasing nutrient use efficiency (Fig. 3). This may occur via a potential re-allocation of nitrogen and phosphorus to optimise photosynthetic capacity (Hasper *et al.*, 2017; Mo *et al.*, 2019). The carboxylation reactions have greater nutrient demand for enzymes, such as RuBisCO, compared to those in the electron transport chain (Evans, 1989; Xu *et al.*, 2012; Raven, 2013). This may allow  $J_{\max}$  to be more plastic and responsive to changes in light availability than  $V_{\max}$ , without

increasing leaf nutrient concentrations. Overall, the reduction in light limitation of photosynthesis in understory trees we observe here could facilitate increased wet-season growth rates as observed previously at this experiment in larger understory trees (Metcalf *et al.*, 2010; Rowland *et al.*, 2015a). Consequently, the ability of small understory trees to respond to light whilst under soil moisture deficit may allow them to regenerate and recover biomass faster than currently predicted by dynamic vegetation models (Fisher *et al.*, 2007).

The ability of small understory trees to respond to increased light availability in the face of drought, increased leaf temperatures and elevated VPD may have various explanations. Firstly, it is possible that despite the reduced soil moisture concentrations (SI Fig. 1), our small trees are less stressed than the adult trees by the drought treatment. This could be a consequence of reduced competition for water following the mortality of large trees (Rowland *et al.*, 2015a), or a result of physiological adjustments to their hydraulic architecture or stomatal conductance. Small trees may be able to avoid drought stress by having stronger stomatal regulation or greater resistance to xylem embolism (Anderegg *et al.*, 2018; Bittencourt *et al.*, 2020). Reduced physiological and architectural constraints also allow small trees to explore more trait combinations than canopy trees that may allow them to be more effective at resisting drought (Reed *et al.*, 2011; Damián *et al.*, 2017). Alternatively, small trees may be able to avoid drought stress by reducing non-maintenance related metabolic activity, such as growth, in the dry season when drought conditions are most pronounced, and maximising growth during the wet season when the soil moisture deficit is reduced. This hypothesis is consistent with previous observations of high wet season and lower dry season diameter growth rates in the trees of smaller size classes on the TFE relative to the control (Metcalf *et al.*, 2010; Rowland *et al.*, 2015a). Whichever mechanism small trees use, our results suggest that these trees are likely to be

able to minimise the impact of the drought, relative to larger trees, in order to facilitate the upregulation of photosynthetic capacity and a release from extreme light limitation.

In addition to upregulation of maximum photosynthetic capacity, we found  $R_{\text{leaf}}$  to increase in small trees following long-term drought by 32% (Fig. 1). These elevated respiratory rates likely reflect the additional maintenance costs of higher photosynthetic capacity under elevated light conditions (Atkin *et al.*, 2015). However, we do not find a direct relationship between  $R_{\text{leaf}}$  and  $V_{\text{cmax}}$  or  $J_{\text{max}}$  in these trees (SI Table 3), suggesting elevated  $R_{\text{leaf}}$  is not simply a consequence of increased photosynthetic capacity. Instead, elevated  $R_{\text{leaf}}$  may reflect a response to increased stress under reduced water availability, potentially representing a means of drought resistance through increasing carbon metabolism for maintenance respiration (Rowland *et al.*, 2018). Under water limitation, particularly when combined with increased irradiance, leaves can accumulate harmful reactive oxygen species (ROS) that cause cell damage (Wang & Vanlerberghe, 2013). An upregulation of respiration can provide a mechanism for leaves to purge these harmful ROS and avoid damage to the photosynthetic metabolic machinery (Atkin & Macherel, 2009). These negative effects of ROS are likely to be strongest during the dry season when water availability is particularly low. Here, small understory trees may have increased their  $R_{\text{leaf}}$  in the dry season to avoid drought stress and facilitate responses to increased light availability. It must also be acknowledged that our results may be influenced by changes in canopy temperature profiles between the plots, with a smaller expected vertical gradient in temperature in the more open canopy of the TFE plot, but measuring this was beyond the scope of this study. We do find large intra-generic variations in  $R_{\text{leaf}}$  and low explanatory power of the TFE treatment in our statistical models (SI Table 2), suggesting this trait is not simply influenced by long-term drought, but also by a range of other unaccounted for factors that are likely species-specific.

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An increase in light availability in the understory of the droughted forest is also likely to be the main explanation for the 15% increase in LMA in small trees on the TFE we observe here. LMA is most strongly controlled by light (Poorter *et al.*, 2009), as leaves invest more in proteins that catalyse photosynthesis and produce more carbohydrates under elevated light conditions (Niinemets *et al.*, 1998; Poorter *et al.*, 2009). Higher concentrations of these high molecular weight metabolites will increase the density of cells and hence LMA. However, it should be noted that elevated LMA may also occur in response to reduced water availability, as a water conservation strategy (Wright *et al.*, 2004; Poorter *et al.*, 2009). Unlike photosynthetic capacity and  $R_{\text{leaf}}$ , LMA has been shown to increase in large trees at the same experiment via increased investment in spongy mesophyll for water storage (Rowland *et al.*, 2015b; Binks *et al.*, 2016; Rowland *et al.* under review), indicating increased LMA in small trees may also be directly associated to lower water availability in the TFE.

We show small trees were more responsive to prolonged drought conditions than large canopy trees, suggesting small trees experience different selection pressures or possess a greater ability to respond to ecosystem level changes in the physical environment. Maximum photosynthetic capacity of small trees increased in response to the TFE treatment – the exact opposite to the response of large trees to the TFE once differences in canopy position have been accounted for in our models (Table 1). In fact, only canopy shading prevents small trees from matching the capacity of large trees. Under prolonged drought, small trees experience changes to both their light and water availability, whereas larger canopy trees will predominantly only experience substantial shifts in water availability. The different responses to prolonged drought suggest small trees are responding most strongly to changes in their light environment, whilst large trees are responding to reductions in soil moisture availability. These shifts may reflect changes in the most limiting resource from the understory to the top canopy,

with understory trees strongly light limited and canopy trees strongly water or nutrient limited (Chazdon & Fetcher, 1984; Sterck & Schieving, 2011).

Despite a general trend of physiological adjustments to prolonged drought conditions in small understory trees, our results clearly demonstrate that different genera have different abilities to respond to changes in their physical environment. Small *Eschweilera* and *Swartzia* trees displayed the greatest directional change in their traits in response to the TFE, as represented by the increase in carbon metabolism trait values ( $J_{\max}$  &  $V_{\max}$ ; Fig. 5). In contrast small *Inga* and *Protium* trees showed very low directional trait variability in response to the TFE. The capacity of a tree to change its traits and potentially acclimate to new environmental conditions is likely to provide a competitive advantage under unstable environmental conditions (Pattison *et al.*, 1998; Lusk *et al.*, 2003), allowing some species to outcompete others. The large positive responses of small *Eschweilera* trees to drought observed here may allow it to maintain its hyperdominance in the Amazon (ter Steege *et al.* 2013), whilst species that show limited responses, e.g. *Protium*, may become less dominant in future communities. However, it should be noted that we show large intrageneric variation in the responses of trees of all sizes (Fig. 5), suggesting taxonomy may not be the only critical factor determining responses to drought-induced changes to the physical environment. Moreover, we focus here on leaf-level physiology and do not measure architectural (e.g. rooting depth) or stomatal responses that may facilitate adaptive responses in genera with low leaf-level plasticity. Further studies investigating recruitment following long-term drought are required to fully understand how taxonomic dominance may change in the future.

## Conclusion



In this study, we highlight the important role of forest structural changes and light in determining physiological responses to long-term drought. Small trees (<10 cm DBH) relative to large trees (>20 cm DBH) display sufficient phenotypic plasticity in leaf morphology and carbon metabolism traits to allow them to respond to increases in light availability despite long-term drought. The ability of small trees to increase their photosynthetic capacity may facilitate increased growth and consequently partial recovery of forest aboveground biomass following earlier drought-induced mortality of large trees (Rowland *et al.*, 2015a). This capacity of small trees to show positive responses to ecosystem-level changes in water and light availability could ultimately allow a more resilient forest to establish and potentially moderate the negative impacts of climate change on the forest ecosystem.

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## **Conflict of Interest statement**

The authors have no conflict of interest to declare.

## References

- Anderegg WRL, Wolf A, Arango-Velez A, Choat B, Chmura DJ, Jansen S, Kolb T, Li S, Meinzer FC, Pita P, et al. 2018. Woody plants optimise stomatal behaviour relative to hydraulic risk. *Ecol Lett* 21(7): 968-977.
- Asner GP, Scurlock JMO, A. Hicke J. 2003. Global synthesis of leaf area index observations: implications for ecological and remote sensing studies. *Global Ecology and Biogeography* 12(3): 191-205.
- Atkin OK, Bloomfield KJ, Reich PB, Tjoelker MG, Asner GP, Bonal D, Bonisch G, Bradford MG, Cernusak LA, Cosio EG, et al. 2015. Global variability in leaf respiration in relation to climate, plant functional types and leaf traits. *New Phytologist* 206(2): 614-636.
- Atkin OK, Macherel D. 2009. The crucial role of plant mitochondria in orchestrating drought tolerance. *Ann Bot* 103(4): 581-597.
- Ayub G, Smith RA, Tissue DT, Atkin OK. 2011. Impacts of drought on leaf respiration in darkness and light in Eucalyptus saligna exposed to industrial-age atmospheric CO<sub>2</sub> and growth temperature. *New Phytologist* 190(4): 1003-1018.
- Bartón K 2018. MuMIn: multi-model inference. R package version 1.42. 1. Retrieved August 15, 2018.
- Bates D, Mächler M, Bolker B, Walker S. 2014. Fitting linear mixed-effects models using lme4. *arXiv preprint arXiv:1406.5823*.
- Bennett AC, McDowell NG, Allen CD, Anderson-Teixeira KJ. 2015. Larger trees suffer most during drought in forests worldwide. *Nature Plants* 1: 15139.
- Binks O, Meir P, Rowland L, da Costa AC, Vasconcelos SS, de Oliveira AA, Ferreira L, Mencuccini M. 2016. Limited acclimation in leaf anatomy to experimental drought in tropical rainforest trees. *Tree Physiol* 36(12): 1550-1561.
- Bittencourt PRL, Oliveira RS, da Costa ACL, Giles AL, Coughlin I, Costa PB, Bartholomew DC, Ferreira LV, Vasconcelos SS, Barros FV, et al. 2020. Amazonia trees have limited capacity to acclimate plant hydraulic properties in response to long-term drought. *Glob Chang Biol* n/a(n/a).
- Bonal D, Burban B, Stahl C, Wagner F, Herault B. 2016. The response of tropical rainforests to drought-lessons from recent research and future prospects. *Ann For Sci* 73(1): 27-44.
- Brown N. 2009. The implications of climate and gap microclimate for seedling growth conditions in a Bornean lowland rain forest. *Journal of Tropical Ecology* 9(2): 153-168.
- Brum M, Vadeboncoeur MA, Ivanov V, Asbjornsen H, Saleska S, Alves LF, Penha D, Dias JD, Aragão LEOC, Barros F, et al. 2018. Hydrological niche segregation defines forest structure and drought tolerance strategies in a seasonal Amazon forest. *Journal of Ecology* 107(1): 318-333.
- Cavaleri MA, Oberbauer SF, Clark DB, Clark DA, Ryan MG. 2010. Height is more important than light in determining leaf morphology in a tropical forest. *Ecology* 91(6): 1730-1739.
- Chazdon RL, Fetcher N. 1984. Photosynthetic Light Environments in a Lowland Tropical Rain Forest in Costa Rica. *The Journal of Ecology* 72(2): 553-564.
- Chazdon RL, Pearcy RW. 1991. The Importance of Sunflecks for Forest Understory Plants. *BioScience* 41(11): 760-766.
- da Costa AC, Galbraith D, Almeida S, Portela BT, da Costa M, Silva Junior Jde A, Braga AP, de Goncalves PH, de Oliveira AA, Fisher R, et al. 2010. Effect of 7 yr of experimental drought on vegetation dynamics and biomass storage of an eastern Amazonian rainforest. *New Phytologist* 187(3): 579-591.

- Damián X, Fornoni J, Domínguez CA, Boege K, Baltzer J. 2017.** Ontogenetic changes in the phenotypic integration and modularity of leaf functional traits. *Functional Ecology* **32**(2): 234-246.
- Damour G, Vandame M, Urban L. 2008.** Long-term drought modifies the fundamental relationships between light exposure, leaf nitrogen content and photosynthetic capacity in leaves of the lychee tree (*Litchi chinensis*). *J Plant Physiol* **165**(13): 1370-1378.
- Damour G, Vandame M, Urban L. 2009.** Long-term drought results in a reversible decline in photosynthetic capacity in mango leaves, not just a decrease in stomatal conductance. *Tree Physiol* **29**(5): 675-684.
- Domingues TF, Meir P, Feldpausch TR, Saiz G, Veenendaal EM, Schrodte F, Bird M, Djangbletey G, Hien F, Compaore H, et al. 2010.** Co-limitation of photosynthetic capacity by nitrogen and phosphorus in West Africa woodlands. *Plant Cell Environ* **33**(6): 959-980.
- Evans JR. 1989.** Photosynthesis and nitrogen relationships in leaves of C3 plants. *Oecologia* **78**(1): 9-19.
- Farquhar GD, von Caemmerer S, Berry JA. 1980.** A biochemical model of photosynthetic CO<sub>2</sub> assimilation in leaves of C3 species. *Planta* **149**(1): 78-90.
- Feldpausch TR, Phillips OL, Brienens RJW, Gloor E, Lloyd J, Lopez-Gonzalez G, Monteagudo-Mendoza A, Malhi Y, Alarcón A, Álvarez Dávila E, et al. 2016.** Amazon forest response to repeated droughts. *Global Biogeochemical Cycles* **30**(7): 964-982.
- Fisher RA, Koven CD, Anderegg WRL, Christoffersen BO, Dietze MC, Farrior CE, Holm JA, Hurtt GC, Knox RG, Lawrence PJ, et al. 2018.** Vegetation demographics in Earth System Models: A review of progress and priorities. *Glob Chang Biol* **24**(1): 35-54.
- Fisher RA, Williams M, da Costa AL, Malhi Y, da Costa RF, Almeida S, Meir P. 2007.** The response of an Eastern Amazonian rain forest to drought stress: results and modelling analyses from a throughfall exclusion experiment. *Global Change Biology* **13**(11): 2361-2378.
- Hartmann H, Adams HD, Hammond WM, Hoch G, Landhäusser SM, Wiley E, Zaehle S. 2018.** Identifying differences in carbohydrate dynamics of seedlings and mature trees to improve carbon allocation in models for trees and forests. *Environmental and Experimental Botany* **152**: 7-18.
- Hasper TB, Dusenke ME, Breuer F, Uwizeye FK, Wallin G, Uddling J. 2017.** Stomatal CO<sub>2</sub> responsiveness and photosynthetic capacity of tropical woody species in relation to taxonomy and functional traits. *Oecologia* **184**(1): 43-57.
- IPCC 2019.** Summary for Policymakers. *Climate Change and Land: an IPCC special report on climate change, desertification, land degradation, sustainable land management, food security, and greenhouse gas fluxes in terrestrial ecosystems.*
- Kamaluddin M, Grace J. 1992.** Photoinhibition and Light Acclimation in Seedlings of *Bischofia javanica*, a Tropical Forest Tree from Asia. *Annals of Botany* **69**(1): 47-52.
- Kitajima K, Cordero RA, Wright SJ. 2013.** Leaf life span spectrum of tropical woody seedlings: effects of light and ontogeny and consequences for survival. *Ann Bot* **112**(4): 685-699.
- Kitajima K, Poorter L. 2008.** Functional basis for resource niche partitioning by tropical trees. *Tropical forest community ecology*: 160-181.
- Krause GH, Virgo A, Winter K. 1995.** High susceptibility to photoinhibition of young leaves of tropical forest trees. *Planta* **197**(4): 583-591.
- Leakey AD, Press MC, Scholes JD. 2003.** Patterns of dynamic irradiance affect the photosynthetic capacity and growth of dipterocarp tree seedlings. *Oecologia* **135**(2): 184-193.

- Lusk CH, Wright I, Reich PB. 2003.** Photosynthetic differences contribute to competitive advantage of evergreen angiosperm trees over evergreen conifers in productive habitats. *New Phytologist* **160**(2): 329-336.
- Malavolta E. 1997.** Avaliação do estado nutricional das plantas: princípios e aplicações/Eurípedes Malavolta, Godofredo Cesar Vitti, Sebastião Alberto de Oliveira.—2. ed., ver. e atual. Piracicaba: Potafos.
- McDowell N, Allen CD, Anderson-Teixeira K, Brando P, Brien R, Chambers J, Christoffersen B, Davies S, Doughty C, Duque A, et al. 2018.** Drivers and mechanisms of tree mortality in moist tropical forests. *New Phytologist* **219**(3): 851-869.
- Meir P, Kruijt B, Broadmeadow M, Barbosa E, Kull O, Carswell F, Nobre A, Jarvis PG. 2002.** Acclimation of photosynthetic capacity to irradiance in tree canopies in relation to leaf nitrogen concentration and leaf mass per unit area. *Plant, Cell and Environment* **25**(3): 343-357.
- Meir P, Mencuccini M, Binks O, da Costa AL, Ferreira L, Rowland L. 2018.** Short-term effects of drought on tropical forest do not fully predict impacts of repeated or long-term drought: gas exchange versus growth. *Philos Trans R Soc Lond B Biol Sci* **373**(1760): 20170311.
- Meir P, Wood TE, Galbraith DR, Brando PM, Da Costa AC, Rowland L, Ferreira LV. 2015.** Threshold Responses to Soil Moisture Deficit by Trees and Soil in Tropical Rain Forests: Insights from Field Experiments. *BioScience* **65**(9): 882-892.
- Metcalfe DB, Lobo-do-Vale R, Chaves MM, Maroco JP, C Aragão LEO, Malhi Y, Da Costa AL, Braga AP, Gonçalves PL, De Athaydes J, et al. 2010.** Impacts of experimentally imposed drought on leaf respiration and morphology in an Amazon rain forest. *Functional Ecology* **24**(3): 524-533.
- Mo Q, Li Za, Sayer EJ, Lambers H, Li Y, Zou B, Tang J, Heskell M, Ding Y, Wang F, et al. 2019.** Foliar phosphorus fractions reveal how tropical plants maintain photosynthetic rates despite low soil phosphorus availability. *Functional Ecology* **33**(3): 503-513.
- Moorcroft PR, Hurtt GC, Pacala SW. 2001.** A Method for Scaling Vegetation Dynamics: The Ecosystem Demography Model (Ed). *Ecological Monographs* **71**(4): 557-586.
- Moore JR, Zhu K, Huntingford C, Cox PM. 2018.** Equilibrium forest demography explains the distribution of tree sizes across North America. *Environmental Research Letters* **13**(8): 084019.
- Mulkey SS, Pearcy RW. 1992.** Interactions between Acclimation and Photoinhibition of Photosynthesis of a Tropical Forest Understorey Herb, *Alocasia macrorrhiza*, during Simulated Canopy Gap Formation. *Functional Ecology* **6**(6): 719-729.
- Nepstad DC, Tohver IM, Ray D, Moutinho P, Cardinot G. 2007.** Mortality of large trees and lianas following experimental drought in an Amazon forest. *Ecology* **88**(9): 2259-2269.
- Newbery DM, Lingensfelder M, Poltz KF, Ong RC, Ridsdale CE. 2011.** Growth responses of understorey trees to drought perturbation in tropical rainforest in Borneo. *Forest Ecology and Management* **262**(12): 2095-2107.
- Niinemets Ü. 2010.** Responses of forest trees to single and multiple environmental stresses from seedlings to mature plants: Past stress history, stress interactions, tolerance and acclimation. *Forest Ecology and Management* **260**(10): 1623-1639.
- Niinemets U, Kull O, Tenhunen JD. 1998.** An analysis of light effects on foliar morphology, physiology, and light interception in temperate deciduous woody species of contrasting shade tolerance. *Tree Physiol* **18**(10): 681-696.
- O'Brien MJ, Reynolds G, Ong R, Hector A. 2017.** Resistance of tropical seedlings to drought is mediated by neighbourhood diversity. *Nat Ecol Evol* **1**(11): 1643-1648.

- O'Brien MJ, Leuzinger S, Philipson CD, Tay J, Hector A. 2014.** Drought survival of tropical tree seedlings enhanced by non-structural carbohydrate levels. *Nature Climate Change* **4**(8): 710-714.
- Pattison RR, Goldstein G, Ares A. 1998.** Growth, biomass allocation and photosynthesis of invasive and native Hawaiian rainforest species. *Oecologia* **117**(4): 449-459.
- Pearcy RW. 1990.** Sunflecks and Photosynthesis in Plant Canopies. *Annual Review of Plant Physiology and Plant Molecular Biology* **41**(1): 421-453.
- Pfeifer M, Kor L, Nilus R, Turner E, Cusack J, Lysenko I, Khoo M, Chey VK, Chung AC, Ewers RM. 2016.** Mapping the structure of Borneo's tropical forests across a degradation gradient. *Remote Sensing of Environment* **176**: 84-97.
- Phillips OL, van der Heijden G, Lewis SL, Lopez-Gonzalez G, Aragao LE, Lloyd J, Malhi Y, Monteagudo A, Almeida S, Davila EA, et al. 2010.** Drought-mortality relationships for tropical forests. *New Phytologist* **187**(3): 631-646.
- Pinheiro J, Bates D, DebRoy S, Sarkar D, Team RC. 2012.** nlme: Linear and nonlinear mixed effects models. *R package version* **3**(0).
- Poorter H, Niinemets U, Poorter L, Wright IJ, Villar R. 2009.** Causes and consequences of variation in leaf mass per area (LMA): a meta-analysis. *New Phytologist* **182**(3): 565-588.
- Poorter L, Hayashida-Oliver Y. 2000.** Effects of seasonal drought on gap and understorey seedlings in a Bolivian moist forest. *Journal of Tropical Ecology* **16**(4): 481-498.
- Quevedo-Rojas A, Garcia-Nunez C, Jerez-Rico M, Jaimez R, Schwarzkopf T. 2018.** Leaf acclimation strategies to contrasting light conditions in saplings of different shade tolerance in a tropical cloud forest. *Funct Plant Biol* **45**(9): 968-982.
- R Core Team. 2013.** R: A language and environment for statistical computing.
- Raven JA. 2013.** Rubisco: still the most abundant protein of Earth? *New Phytologist* **198**(1): 1-3.
- Reed TE, Schindler DE, Waples RS. 2011.** Interacting effects of phenotypic plasticity and evolution on population persistence in a changing climate. *Conserv Biol* **25**(1): 56-63.
- Reynolds-Henne CE, Langenegger A, Mani J, Schenk N, Zumsteg A, Feller U. 2010.** Interactions between temperature, drought and stomatal opening in legumes. *Environmental and Experimental Botany* **68**(1): 37-43.
- Rowland L, da Costa AC, Galbraith DR, Oliveira RS, Binks OJ, Oliveira AA, Pullen AM, Doughty CE, Metcalfe DB, Vasconcelos SS, et al. 2015a.** Death from drought in tropical forests is triggered by hydraulics not carbon starvation. *Nature* **528**(7580): 119-122.
- Rowland L, da Costa ACL, Oliveira AAR, Oliveira RS, Bittencourt PL, Costa PB, Giles AL, Sosa AI, Coughlin I, Godlee JL, et al. 2018.** Drought stress and tree size determine stem CO<sub>2</sub> efflux in a tropical forest. *New Phytologist* **218**(4): 1393-1405.
- Rowland L, Lobo-do-Vale RL, Christoffersen BO, Melem EA, Kruijt B, Vasconcelos SS, Domingues T, Binks OJ, Oliveira AA, Metcalfe D, et al. 2015b.** After more than a decade of soil moisture deficit, tropical rainforest trees maintain photosynthetic capacity, despite increased leaf respiration. *Glob Chang Biol* **21**(12): 4662-4672.
- Ruger N, Wirth C, Wright SJ, Condit R. 2012.** Functional traits explain light and size response of growth rates in tropical tree species. *Ecology* **93**(12): 2626-2636.
- Schneider CA, Rasband WS, Eliceiri KW. 2012.** NIH Image to ImageJ: 25 years of image analysis. *Nat Methods* **9**(7): 671-675.



- Schuldt B, Leuschner C, Horna V, Moser G, Köhler M, van Straaten O, Barus H. 2011. Change in hydraulic properties and leaf traits in a tall rainforest tree species subjected to long-term throughfall exclusion in the perhumid tropics. *Biogeosciences* **8**(8): 2179-2194.
- Sharkey TD, Bernacchi CJ, Farquhar GD, Singsaas EL. 2007. Fitting photosynthetic carbon dioxide response curves for C(3) leaves. *Plant Cell Environ* **30**(9): 1035-1040.
- Smith B, Prentice IC, Sykes MT. 2008. Representation of vegetation dynamics in the modelling of terrestrial ecosystems: comparing two contrasting approaches within European climate space. *Global Ecology and Biogeography* **10**(6): 621-637.
- Stahl C, Herault B, Rossi V, Burban B, Brechet C, Bonal D. 2013. Depth of soil water uptake by tropical rainforest trees during dry periods: does tree dimension matter? *Oecologia* **173**(4): 1191-1201.
- Sterck F, Markesteijn L, Toledo M, Schieving F, Poorter L. 2014. Sapling performance along resource gradients drives tree species distributions within and across tropical forests. *Ecology* **95**(9): 2514-2525.
- Sterck F, Schieving F. 2011. Modelling functional trait acclimation for trees of different height in a forest light gradient: emergent patterns driven by carbon gain maximization. *Tree Physiol* **31**(9): 1024-1037.
- van de Weg MJ, Meir P, Grace J, Ramos GD. 2012. Photosynthetic parameters, dark respiration and leaf traits in the canopy of a Peruvian tropical montane cloud forest. *Oecologia* **168**(1): 23-34.
- Wang J, Vanlerberghe GC. 2013. A lack of mitochondrial alternative oxidase compromises capacity to recover from severe drought stress. *Physiol Plant* **149**(4): 461-473.
- Warton DI, Duursma RA, Falster DS, Taskinen S. 2012. smatr 3- an R package for estimation and inference about allometric lines. *Methods in Ecology and Evolution* **3**(2): 257-259.
- Wright IJ, Reich PB, Westoby M, Ackerly DD, Baruch Z, Bongers F, Cavender-Bares J, Chapin T, Cornelissen JH, Diemer M, et al. 2004. The worldwide leaf economics spectrum. *Nature* **428**(6985): 821-827.
- Xu C, Fisher R, Wullschlegel SD, Wilson CJ, Cai M, McDowell NG. 2012. Toward a mechanistic modeling of nitrogen limitation on vegetation dynamics. *PLoS One* **7**(5): e37914.
- Zuur A, Ieno EN, Walker N, Saveliev AA, Smith GM. 2009. *Mixed effects models and extensions in ecology with R*: Springer Science & Business Media.

## Figures captions

Figure 1: Boxplots showing how maximum photosynthetic capacity ( $J_{\max}$ : a;  $V_{\max}$ : b), leaf dark respiration ( $R_{\text{leaf}}$ : c), leaf mass per area (LMA: d), leaf nitrogen ( $[N]_{\text{leaf}}$ : e) and leaf phosphorus ( $[P]_{\text{leaf}}$ : f) differed between the control and TFE plot for small understory trees (1-10 cm DBH). Asterisks represent significant differences from linear mixed effect model tests at different p-values (ns: non-significant; \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ). See SI Table 2 for full model details. Boxes represent the interquartile range with a horizontal line for the median and the whiskers represent 1.5\*interquartile range or the maximum and minimum point. Dots represent points outside the extent of the whiskers.

Figure 2: Standardised Major Axis (SMA) regression between  $J_{\max}$  and  $V_{\max}$  on a  $\log_{10}$  scale for small understory trees (1-10 cm DBH) on the control plot (black) and the TFE plot (grey). The dashed line represents a 1:1 ratio. A significant difference in the elevation ( $p < 0.01$ ) and a shift in the data ( $p < 0.001$ ) between the treatments were found, but no significant difference between the slopes was found ( $p = 0.757$ ).

Figure 3: Boxplots showing how  $J_{\max}$  (a, b),  $V_{\max}$  (c, d) and  $R_{\text{leaf}}$  (e, f) nutrient use efficiency (NUE) changed between the control and TFE treatment for nitrogen (a, c, e) and phosphorus (b, d, f). NUE was calculated by dividing gas exchange parameters on a mass basis by leaf nutrient concentrations. Asterisks represent significant differences from linear mixed effects models between the two treatments (ns: non-significant; \*  $p < 0.05$ ; \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$ ). Boxes represent the interquartile range with a horizontal line for the median and the whiskers represent 1.5\*interquartile



range or the maximum and minimum point. Dots represent points outside the extent of 1.5\*interquartile range.

Figure 4: Boxplots showing how  $J_{\max}$  (a),  $V_{\max}$  (b),  $R_{\text{leaf}}$  (c) and leaf mass per area (LMA; d) change between the control (blue) and TFE (red) treatments for large (>20 cm DBH; red and dark blue) and small (1-10 cm DBH; pink and light blue) trees. Identical letters represent categories where there is no significant difference ( $p > 0.05$ ) from pairwise Wilcoxon rank-sum tests. Boxes represent the interquartile range with a horizontal line for the median and the whiskers represent 1.5\*interquartile range or the maximum and minimum point. Dots represent points outside the extent of 1.5\*interquartile range.

Figure 5: Violin plots comparing  $J_{\max}$  (a),  $V_{\max}$  (b),  $R_{\text{leaf}}$  (c) and leaf mass per area (LMA; d) between the Control (C) and TFE (T) treatment for large (>20 cm DBH) and small (1-10 cm DBH) trees across the five most common genera: *Eschweilera*, *Inga*, *Licania*, *Protium*, *Swartzia*. All genera with  $n \geq 2$  for each of the four categories are presented. White dots represent the median, thick grey lines represent the interquartile range and thin grey lines represent the mean  $\pm 1.5$  standard errors.

## Tables

Table 1: Parameter estimates for the minimal adequate models explaining  $J_{\max}$ ,  $V_{\max}$ ,  $R_{\text{leaf}}$ , leaf mass per area (LMA) and leaf thickness for large (>20 cm DBH;  $n = 61$ ) and small (1-10 cm DBH;  $n = 48$ ) trees. The intercept represents the trait value when the fixed effects are zero: large control trees that are not shaded. Canopy position represents the difference when a tree is shaded, plot represents the difference between the TFE treatment compared to the control and tree size represents the difference for small trees compared to large trees. Coefficient estimates  $\pm$  the standard error are presented for each fixed effect and refer to the differences between the factor levels indicated in brackets for each column and the overall intercept. The genus intercept is given whenever the genus included as a random intercept effect improved the model fit. Species nested within genus was not significant for any traits, so is not included here. Random effect variance for genus  $\pm$  standard deviation is presented. Total (conditional)  $R^2$  represents the total variation explained by the model and is partitioned into the variation explained by the fixed effects (marginal  $R^2$ ) and fixed plus random-effects (conditional  $R^2$ ). Asterisks represent the significance level of each variable: ns  $P > 0.05$ ; \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ . Dashes represent variables that were not retained in the minimal adequate model but were tested in the initial fully saturated model. See Methods and SI Table 3 for more details about model selection.

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Trait	Intercept	Canopy Position (shaded)	Plot (TFE)	Tree Size (small)	Size : Canopy interaction (TFE : shaded)	Size : Plot interaction (TFE : small)	Genus intercept	Fixed Effect (marginal) R <sup>2</sup>	Total (conditional) R <sup>2</sup>
<i>J</i> <sub>max</sub>		***	*	***	-	***	*		
	55.68 ± 2.93	-14.90 ± 3.29	-7.52 ± 3.01	-18.82 ± 3.84		18.88 ± 4.56	20.28 ± 4.50	0.526	0.596
<i>V</i> <sub>cmax</sub>		**	ns	***	-	**	**		

	29.96 ± 1.83	-5.82 ± 1.89	-3.27 ± 1.72	-7.59 ± 2.18		6.69 ± 2.58	10.70 ± 3.27	0.336	0.482
<b><i>R</i><sub>leaf</sub></b>		-	ns	***	-	-	-		
	0.63 ± 0.04		0.09 ± 0.05	-0.23 ± 0.05				0.188	0.188
<b>LMA</b>		-	*	***	-	-	***		
	103.91 ± 6.81		8.19 ± 4.10	-30.66 ± 4.60			266.90 ± 16.34	0.267	0.527
<b>Leaf thickness</b>	0.18 ± 0.01	-	-	-	-	-	**		
							0.0005 ± 0.022	0	0.232











